

Infinium™ MethylationEPIC BeadChip

Affordable methylome analysis meets cutting edge content.

Highlights

- **Comprehensive coverage of coding regions and enhancers**

Over 850,000 methylation sites per sample at single-nucleotide resolution

- **High assay reproducibility**

>98% reproducibility for technical replicates

- **Simple workflow**

PCR-free protocol with the powerful Infinium HD Assay

- **Compatible with FFPE samples**

Protocol available for methylation studies on formalin-fixed, paraffin-embedded (FFPE) samples

Introduction

DNA methylation plays an important and dynamic role in regulating gene expression. It allows cells to acquire and maintain a specialized state, suppresses the expression of viral and nonhost DNA elements, and facilitates response to environmental stimuli. Aberrant DNA methylation (hyper- or hypomethylation) and its impact on gene expression have been implicated in many disease processes, including cancer, neurological disorders, aging, and development.^{1,2}

To enable cost-effective DNA methylation analysis for various applications, Illumina offers a robust methylation profiling platform consisting of proven chemistries and the iScan and NextSeq 550 Systems. The past five years of methylome research, including projects like ENCODE and FANTOM5, have identified enhancer regions as critical sites for differential methylation. The Infinium MethylationEPIC BeadChip (Figure 1) builds on the Infinium HumanMethylation450 BeadChip with >90% of the original CpGs plus an additional 350,000 CpGs in enhancer regions. By providing quantitative methylation measurement at the single-CpG-site level for normal and formalin-fixed paraffin-embedded (FFPE) samples, this assay offers powerful resolution for understanding epigenetic changes.

Comprehensive genome-wide coverage

The Infinium MethylationEPIC BeadChip provides exceptional coverage of CpG islands, RefSeq genes, ENCODE open chromatin, ENCODE transcription factor binding sites, and FANTOM5 enhancers (Figure 2). Infinium HD technology enables content selection independent of bias-associated limitations often associated with methylated DNA capture methods.

Importantly, the Infinium MethylationEPIC BeadChip contains >90% of the original Infinium Methylation450 BeadChip content, chosen to provide a broad, comprehensive view of the methylome.

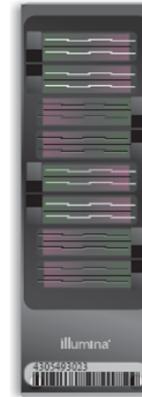


Figure 1: The Infinium MethylationEPIC BeadChip—The Infinium MethylationEPIC BeadChip features > 850,000 CpGs in enhancer regions, gene bodies, promoters, and CpG islands.

The Infinium MethylationEPIC BeadChip targets regions identified by the ENCODE project as potential enhancers. ENCODE tracks for open chromatin, FAIRE assays, and transcription factor binding sites were overlaid to identify the most dynamic regions of the genome for content (Figure 3). Finally, the array targets enhancers identified by the FANTOM5 project across tissue types. The result is a pan-enhancer and coding region view of the methylome that can be used for epigenome-wide association studies on various human tissues and includes the following content categories requested by methylation experts:

- CpG sites outside of CpG islands
- Non-CpG methylated sites identified in human stem cells (CHH sites)
- Differentially methylated sites identified in tumor versus normal (multiple forms of cancer) and across several tissue types
- FANTOM5 enhancers
- ENCODE open chromatin and enhancers
- DNase hypersensitivity sites
- miRNA promoter regions
- >90% of content on the Illumina HumanMethylation450 BeadChip

Streamlined workflow

The Infinium MethylationEPIC BeadChip follows a user-friendly, streamlined workflow that does not require PCR. Its low sample input requirement (as low as 250 ng), enables analysis of valuable samples derived from limited DNA sources. Infinium MethylationEPIC BeadChip kits contain all required reagents for performing methylation analyses (except for the bisulfite conversion kit, which is available separately).

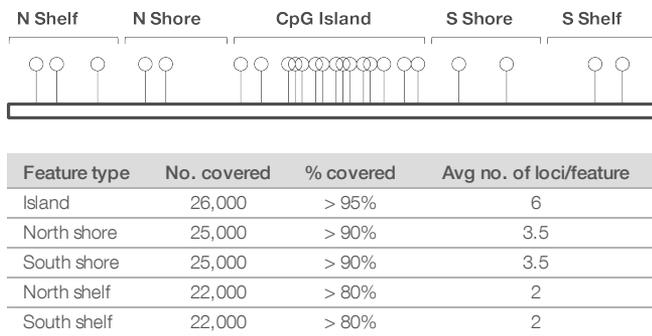


Figure 2: The Infinium MethylationEPIC BeadChip provides dense coverage throughout gene regions—The Infinium MethylationEPIC BeadChip offers broad coverage across gene regions, CpG islands/CpG island regions, shelves, and shores for a comprehensive view of the methylation state.

High-quality data

The Infinium MethylationEPIC BeadChip applies both Infinium I and II assay chemistry technologies (Figure 4) to enhance the depth of coverage for methylation analysis. The addition of the Infinium II design allows use of degenerate oligonucleotide probes for a single bead type. This enables each of up to three underlying CpG sites to be either methylated or unmethylated with no impact on the result for the queried site. Illumina scientists rigorously test every product to ensure strong and reproducible performance.

Reproducibility

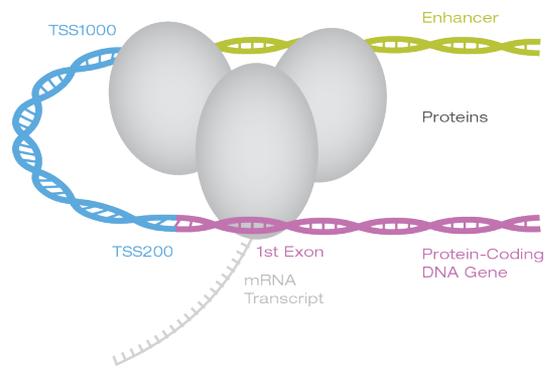
Reproducibility has been determined based on the correlation of results generated from technical replicates. The Infinium MethylationEPIC BeadChip shows strong correlation between replicates ($r^2 > 0.98$), and with the HumanMethylation450 BeadChip (Figure 6A and 6B). The Infinium HumanMethylation450 BeadChip also shows a high R2 correlation with whole genome bisulfite sequencing data (Figure 6C).

Analytical sensitivity

By comparing the results of replicate experiments (duplicates of eight biological samples), Illumina scientists have shown that the Infinium MethylationEPIC BeadChip reliably detects a delta-beta value of 0.2 with a lower than 1% false positive rate.

Internal quality controls

Infinium HD assays produce high-quality data with sample-dependent and independent controls. The Infinium MethylationEPIC BeadChip includes negative controls to account for decreased sequence complexity after bisulfite conversion. The GenomeStudio™ Methylation Module Software has an Integrated Controls Dashboard for easy monitoring of controls. For large-scale studies, the BeadArray Controls Reporter outputs a simple-to-scan excel file for rapid analysis of multiple controls.



Feature type	No. of features mapped	% features covered	Avg no. of loci/feature
RefSeq			
NM_TSS200 ^a	> 33,000	> 85%	3
NM_TSS1500	> 38,000	> 97%	5
NM_5'UTR	> 31,000	> 83%	7
NM_1stExon	> 33,000	> 84%	2
NM_3'UTR	> 27,000	> 69%	1
NM_ExonBoundaries	> 36,000	> 96%	0.5
NR_TSS200	> 7000	> 65%	1
NR_TSS1500	> 9000	> 84%	3
NR_ExonBoundaries	> 8000	> 77%	0.2
GenCode Basic v12			
TSS200	> 114,000	> 84%	2
TSS1500	> 130,000	> 87%	5
5'UTR	> 83,000	> 82%	7
First Exon	> 75,000	> 57%	2
3'UTR	> 63,000	> 72%	3
Exon Boundaries	> 10,000	> 30%	0.5
Enhancers			
ENCODE Open Chromatin ^b Evidence ≥ 4	> 153,000	> 66%	2
ENCODE TFBS in Open Chromatin ^c Evidence ≥ 3	> 166,000	> 72%	1
ENCODE TFBS in Open Chromatin Evidence ≥ 4	> 142,000	> 78%	3
FANTOM5 Enhancers ^d	> 23,000	> 83%	1

- a. Distance in base pairs from transcriptional start site (TSS).
- b. ENCODE Open Chromatin: Genomic regions ≥ 4 pieces of evidence supporting open chromatin status from ENCODE tracks within DNase1_FAIRE_ChIP_Synthesis_from_ENCODE_OpenChrom_Duke_UNC_UTA.
- c. ENCODE TFBS in Open Chromatin: Regions of ENCODE Open Chromatin defined above also identified as transcription factor binding sites (TFBS) in the track TFBS_PeakSeq-based_Peaks. Data are provided with ≥ 3 and ≥ 4 pieces of evidence, as indicated.
- d. Genomic regions identified as enhancers by the FANTOM5 project.

Figure 3: The Infinium MethylationEPIC BeadChip provides dense coverage throughout enhancer regions—The Infinium MethylationEPIC BeadChip offers dense coverage across multiple content categories requested by methylation experts including enhancer regions.

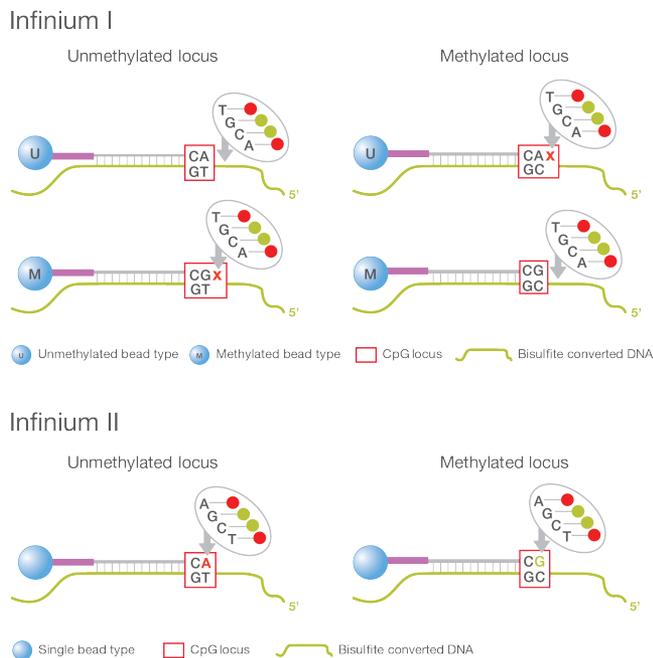


Figure 4: Broad coverage using Infinium I and II assay designs—The Infinium MethylationEPIC BeadChip employs both Infinium I and Infinium II assays. Infinium I assay design employs two bead types per CpG locus, one each for the methylated and unmethylated states. The Infinium II design uses one bead type, with the methylated state determined at the single base extension step after hybridization.

Integrated analysis software

Infinium MethylationEPIC BeadChip data analysis is supported by the GenomeStudio Methylation Module, enabling researchers to perform differential methylation analysis for small scale studies. GenomeStudio software version 2011.1 features advanced visualization tools that enable researchers to view vast amounts of data in a single graph, such as heat maps, scatter plots, and line plots (Figure 5).

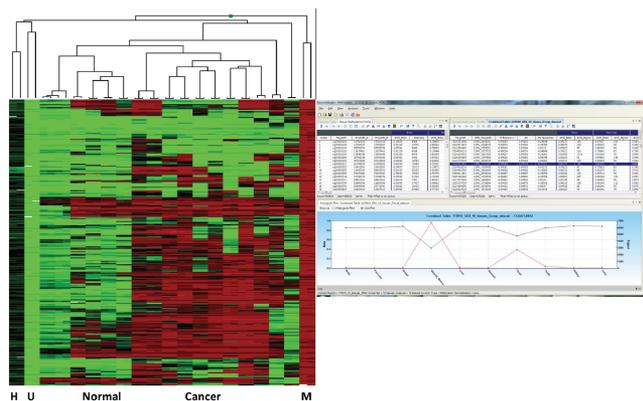


Figure 5: Integrated Data Analysis with Illumina GenomeStudio Software—GenomeStudio software supports DNA methylation analysis on any platform. Data are displayed in intuitive graphics (heat map, left). Gene expression data can be easily integrated with methylation projects (plotted on right).

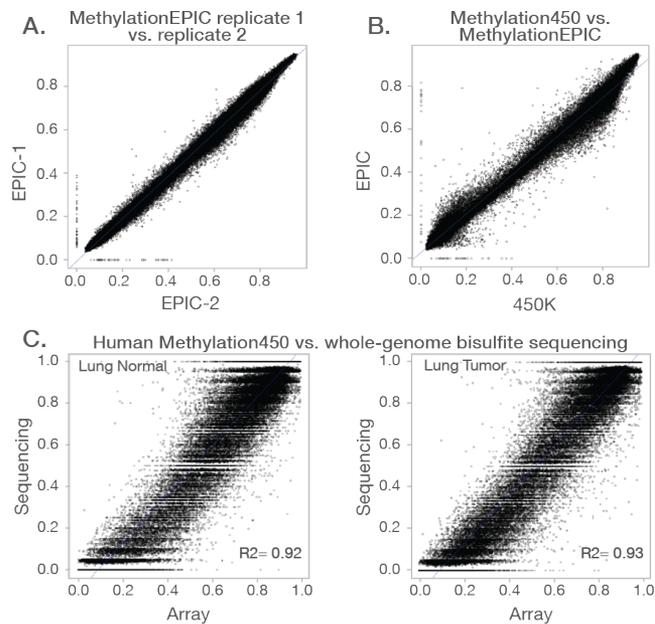


Figure 6: Infinium methylation technology shows high reproducibility and correlation to sequencing data—(A) Technical replicates of beta values for noncancer samples on the Infinium MethylationEPIC BeadChip show a > 98% R2 value. (B) Comparison of beta values for noncancer samples on overlapping content between the Infinium HumanMethylation450 BeadArray vs the Infinium MethylationEPIC BeadChip show a > 98% R2 value. (C) Infinium HumanMethylation450K data show high methylation call correlation when compared to whole-genome bisulfite sequencing data across normal and tumor lung tissues.

High compatibility with other software

These tools and the GenomeStudio Genome Browser display valuable information such as chromosomal coordinates, percent GC, location in a CpG Island, and methylation b values. For large-scale studies, there are many freely available analysis packages that work in the software framework R for normalization and differential analysis of methylation data.^{3,4}

High cross-application compatibility

Data generated by the Infinium HD methylation assay are easily compatible with data from other Illumina applications, including gene expression profiling. This enables researchers to perform cross application analysis such as the integration of gene expression data with Infinium MethylationEPIC BeadChip methylation data.

Methylation studies with FFPE samples

Methylation studies on FFPE samples are enabled with a modified version of the Infinium MethylationEPIC BeadChip protocol.⁵ This protocol harnesses the Infinium FFPE DNA Restoration Solution⁶ to produce robust, reproducible results (Table 1). The Illumina FFPE QC and the Infinium HD FFPE DNA Restore Kits are included. The FFPE DNA Restore Solution and MethylationEPIC BeadChip kits are the same for normal and FFPE samples. Investigators running

FFPE samples should only follow the workflow described in the Infinium HD FFPE Methylation Assay protocol (manual or automated),⁶⁻⁸ as it includes important changes to the standard protocols for each kit.

Table 1: Comparative Infinium methylation data quality metrics standard vs. FFPE

Infinium MethylationEPIC BeadChip	Standard protocol	FFPE protocol
Reproducibility (technical replicates)	$r^2 \geq 98\%$	$r^2 \geq 98\%$
Number of sites detected ^a	$\geq 96\%$	$\geq 90\%$

a. Based on noncancer samples, recommended sample input amounts of high-quality DNA as confirmed by PicoGreen and following all other Illumina recommendations as per User Guides.

Synergy with TruSeq™ Methyl Capture EPIC

The panel included in the TruSeq Methyl Capture EPIC Library Prep Kit, an enrichment-based bisulfite sequencing method, mirrors and expands upon the content of the Infinium MethylationEPIC BeadChip. Researchers can move easily between array and sequencing technologies, taking advantage of the low price point on the Infinium MethylationEPIC BeadChip for large-scale screens and diving deep on specific samples or subsets with the TruSeq Methyl Capture EPIC Library Prep Kit. These combined technologies represent the best of both worlds of methylation analysis.

Summary

The Infinium MethylationEPIC BeadChip offers a unique combination of comprehensive, expert-selected coverage, high sample throughput capacity, and affordable price that makes it an ideal solution for large sample-size, genome-wide DNA methylation studies.

Ordering Information

Product	Description	Catalog No.
Infinium MethylationEPIC BeadChip Kit (16 Samples)	Each package contains 2 BeadChips and reagents for analyzing DNA methylation in 16 human DNA samples.	WG-317-1001
Infinium MethylationEPIC BeadChip Kit (32 Samples)	Each package contains 4 BeadChips and reagents for analyzing DNA methylation in 32 human DNA samples.	WG-317-1002
Infinium MethylationEPIC BeadChip Kit (96 Samples)	Each package contains 12 BeadChips and reagents for analyzing DNA methylation in 96 human DNA samples.	WG-317-1003

Each Infinium MethylationEPIC BeadChip can process eight samples in parallel and assay > 850,000 methylation sites per sample.

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Learn More

To learn more about the product, visit www.illumina.com/products/by-type/microarray-kits/infinium-methylation-epic.html

References

- Portela A, Esteller M. *Epigenetic modifications and human disease*. *Nat Biotech*. 2010;28:1057-1068.
- Rakyan VK, Down TA, Balding DJ, Beck S. *Epigenome-wide association studies for common human diseases*. *Nat Rev Genetics*. 2011;12(8):529-541.
- Morris TJ, Butcher LM, Feber A, et al. *ChAMP: 450K chip analysis methylation pipeline*. *Bioinformatics*. 2014;30(3):428-430.
- Assenov Y, Muller F, Lutsik P, et al. *Comprehensive analysis of DNA methylation with RnBeads*. *Nat Methods*. 2015;11(11):1138-1140.
- Illumina (2015) *Infinium HD FFPE Methylation Assay Manual Protocol*. Accessed September 28, 2018.
- Illumina. (2011) *Infinium HD FFPE DNA Restoration Protocol*. Accessed September 28, 2018.
- Illumina (2015) *Infinium HD FFPE Methylation Assay, Automated Protocol EUC*. Accessed September 28, 2018.
- Illumina (2018) *Illumina FFPC QC Assay Protocol*. Accessed September 28, 2018.